

Amendments to the Claims

This listing of claims replaces all prior versions and listings of claims in the application.

Listing of Claims

1. (Canceled)
2. (Currently Amended) A method of producing amplified RNA (aRNA), said method comprising
 - a) reverse transcribing an RNA template using a first promoter-primer oligonucleotide comprising a first primer sequence and a first promoter sequence and an RNA dependent DNA polymerase to produce a first strand cDNA comprising the first promoter sequence in a reaction [[which]] that is completed in 45 minutes or less;
 - b) producing a second strand cDNA complementary to said first strand cDNA using a DNA dependent polymerase, in the presence of random primers to prime synthesis of said second strand cDNA, in a reaction [[which]] that is completed in 45 minutes or less, to thereby produce a double stranded cDNA comprising the first promoter sequence; and
 - c) producing amplified RNA from the double stranded cDNA by in vitro transcription using a DNA dependent RNA polymerase [[which]] that initiates transcription from the first promoter sequence in a reaction [[which]] that is completed in 180 minutes or less;wherein after b) and before c), or after c), the product produced by b) or c) is purified by contacting said product with a solid phase [[which]] that binds nucleic acids followed by eluting bound nucleic acids from said solid phase in an elution volume of less than 50 microliters.
3. (Previously Presented) The method of claim 2 wherein said RNA template is mRNA.
4. (Previously Presented) The method of claim 2 wherein said RNA template is in a cellular mRNA preparation.

5. (Previously Presented) The method of claim 2 wherein the first primer sequence comprises an oligo or poly dT sequence.

6. (Original) The method of claim 5 wherein said oligo or poly dT sequence is at least about eight dT in length.

7. (Previously Presented) The method of claim 2 wherein said random primers are six nucleotides or longer in length.

8. (Original) The method of claim 7 wherein said random primers are nine nucleotides or longer in length.

9. (Previously Presented) The method of claim 2 wherein the bound nucleic acids are eluted in an elution volume of 25 microliters or less.

10. (Original) The method of claim 9 wherein said elution volume is 15 microliters or less.

11. (Previously Presented) The method of claim 2 wherein the first promoter sequence comprises a T7 promoter sequence.

12. (Previously Presented) The method of claim 2 wherein said solid phase is a filter.

13. (Previously Presented) The method of any one of claims 2 or 12 wherein said solid phase comprises silica.

14. (Original) The method of claim 13 wherein said solid phase is glass powder, silica particles, glass fibers or microfibers, diatomaceous earth, or borosilicate glass.

15. (Original) The method of any one of the preceding claims wherein the wetting capacity of the solid phase is approximately the same as, or less than, the elution volume.

16. (Original) The method of any one of the preceding claims wherein said eluting of bound nucleic acids comprises centrifugation.

17. (Original) The method of claim 16 wherein said centrifugation is in two steps.

18. (Original) The method of claim 17 wherein said two steps comprise a first step and a second step at a higher speed than said first step.

19. (Original) The method of claim 16 wherein said centrifugation is without the application of a vacuum.

20. (Currently Amended) The method of claim 2 wherein a) or b) is conducted in a reaction [[which]] that is completed in 25 minutes or less.

21. (Currently Amended) The method of claim 2, wherein the amplified RNA is further amplified by a method comprising

d) reverse transcribing said amplified RNA using random primers and [[a DNA]] an RNA dependent DNA polymerase in a reaction [[which]] that is completed in 45 minutes or less to produce a first strand cDNA;

e) producing a second strand cDNA complementary to said first strand cDNA using a second promoter-primer oligonucleotide comprising a second primer sequence and a second promoter sequence and a DNA dependent DNA polymerase in a reaction [[which]] that is

completed in 45 minutes or less, to thereby produce a double stranded cDNA comprising the second promoter sequence; and

f) producing re-amplified RNA from the double stranded cDNA by in vitro transcription using a DNA dependent RNA polymerase [[which]] that initiates transcription from the second promoter sequence;

wherein after e) and before f) or after f), the product produced by e) or f) is purified by contacting said product with a solid phase [[which]] that binds nucleic acids followed by eluting bound nucleic acids from said solid phase in an elution volume of less than 50 microliters.

22. (Previously Presented) The method of claim 21 wherein the second promoter sequence comprises a T3 or SP6 promoter sequence.

23. (Original) The method of claim 21 wherein said random primers are six nucleotides or longer in length.

24. (Original) The method of claim 23 wherein said random primers are nine nucleotides or longer in length.

25. (Previously Presented) The method of claim 21 wherein said second primer sequence comprises a known primer sequence.

26. (Original) The method of claim 25 wherein said known primer sequence is complementary to the 3' region of said amplified RNA.

27. (Previously Presented) The method of claim 21, 25, or 26 wherein said second promoter-primer oligonucleotide comprises a T3 or SP6 promoter region.

28. (Previously Presented) The method of claim 21 wherein the elution of bound nucleic acids after e) or f) is in an elution volume of 25 microliters or less.

29. (Original) The method of claim 28 wherein said elution volume is 15 microliters or less.

30. (Previously Presented) The method of any one of claims 21-29 wherein the solid phase is a filter.

31. (Previously Presented) The method of any one of claims 21-30 wherein said solid phase comprises silica.

32. (Original) The method of claim 31 wherein said solid phase is glass powder, silica particles, glass fibers or microfibers, diatomaceous earth, or borosilicate glass.

33. (Previously Presented) The method of any one of claims 21-32 wherein the wetting capacity of the solid phase is approximately the same as, or less than, the elution volume.

34. (Original) The method of any one of claims 21-32 wherein said eluting of bound nucleic acids comprises centrifugation.

35. (Original) The method of claim 34 wherein said centrifugation is in two steps.

36. (Original) The method of claim 35 wherein said two steps comprise a first step and a second step at a higher speed than said first step.

37. (Original) The method of claim 34 wherein said centrifugation is without the application of a vacuum.

Applicant : Erlander *et al.*
Serial No. : 10/507,932
Filed : January 9, 2006
Page : 9 of 13

Attorney Docket No.: 26370-0052US1
Client Ref. No.: LT00240 US

38. (Currently Amended) The method of claim 21 wherein d) or e) is conducted in a reaction [[time of]] that is completed in 25 minutes or less.